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AN ATTEMPT TO PRODUCE AN ATYPICAL EPITHELIAL GROWTH BY INJECTION OF SCHARLACH R. IN OLIVE OIL; A CONTROL OF FISCHER'S EXPERIMENTS.*

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PERHAPS no work bearing on the etiology of carcinoma has been more suggestive and has caused more comment than that recently reported from Ribbert's laboratory by Dr. B. Fischer.¹ On account of the important bearing his results may have on our ideas of the origin of these malignant growths it seemed desirable that the work be repeated by other observers. Accordingly, at the suggestion of Dr. Warthin, I have attempted to do this.

After having reviewed the previous attempts to produce a destructive atypical epithelial proliferation by means of irritation, and concluding that no progress was to be made in that direction, Fischer was led by consideration of the work of Loeb on parthenogenesis to ask if he could not, by producing a proper physico-chemical environment, induce an epithelial proliferation entirely analogous to that produced by Loeb in the unfertilized egg of the sea-urchin. Ribbert had previously pointed out that in carcinomatous growths arising from the skin there is often an inflammatory infiltration of the sub-epithelial connective tissue, and Fischer therefore thought to bring about his desired condition by producing a chronic inflammation of the papillary bodies. He accordingly implanted bits of sterile muscle, liver, spleen, and other tissues beneath the epithelium of rabbits' ears. The only result was a slight thickening of the epithelium when the inflammatory reaction reached the germ layer. He then tried injecting agar-agar, with and without calcium salts, and in one case found that the epithelium did proliferate, and grew down around the agar masses. Further experiments upon other animals and upon the same animal failed to give like results, and he concluded that in the

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¹ *Munch. med. Wchnschr.*, 1906, 53, p. 2041.

one apparently successful case some epithelial cells had been carried in with the injecting needle, and in their new location had proliferated. Next he used olive oil, on account of the slowness with which it is absorbed, the slight chronic inflammation which it produces, and the ease with which it can be made to permeate the tissues. The pure oil caused, after several weeks, a thickening of the epithelium, and in some cases the development of short processes, but no atypical growth. Various substances were added to the oil without further result until the fat-staining dyes Scharlach R. Sudan III, and Indo-phenol were used.

According to Fischer, when a saturated solution of Scharlach R., in pure olive oil, which he calls "scarlet oil," was caused to permeate the tissues beneath the epithelium a marked change followed. The tissues became hyperemic, infiltrated with round cells, connective-tissue giant cells were formed, and there developed a myxomatous-like connective tissue. He further states that within a few days the superficial epithelium, the cells of the rete Malpighii, and those of the hair follicles and gland ducts showed mitoses, typical and atypical; that processes of epithelium grew down into the subjacent tissue toward the oil droplets, and in many cases came to surround them: The picture so produced was, in his opinion, to be distinguished in no way histologically from a squamous-celled carcinoma in man. He says that in some cases the cartilage was invaded, and that in one case he found an epithelial plug growing in a lymph channel.

He thought the growth was independent of the inflammatory reaction since the processes penetrated beneath the inflammatory zone, and that it was not the result of irritation since neither olive oil alone nor other irritants produced the same effect, and since no such result followed repeated painting of the rabbit's ear with scarlet oil through a period of several months.

So long as the tissues contained scarlet oil the growth continued, but when this was exhausted growth ceased and the epithelial plugs underwent cornification, resulting in cholesteatomatous masses which broke and discharged outward.

He states that he obtained the same though less marked results by the use of dry Scharlach R.

In general, within three weeks after injection, visible growth could

be seen over the area of injection. Drawings are shown of tissue excised after seven to 24 days, one injection having been made in some cases, two in others.

Fischer sees in these results the evidence of a chemotactic influence of the scarlet oil on the epithelial cells, and suggests that certain chemical groups, which he calls "attraxines," exist in the scarlet oil, and that these attraxines, which are specific in their influence, may under certain conditions develop in the body and give rise to carcinomatous proliferation of the epithelium. He promises further reports, and expresses the hope that in the meantime other workers will confirm his observations.

If these observations can be confirmed and the attraxine theory placed on a firm basis, it would without doubt go far toward explaining the etiology of carcinoma, and probably of other malignant tumors. It therefore seemed important that control experiments be done, and accordingly immediately after the appearance of Fischer's paper, work to that end was begun in this laboratory.

Unfortunately Fischer gives no detailed account of his technic, so that in repeating the experiments I could follow only the bare outlines of his methods.

The work done here is as follows: Six white rabbits were used, three about two months old and three full grown. A saturated solution of Scharlach R. in olive oil was made up, the Scharlach R. used having been obtained in Berlin from E. Leitz, the oil being Lucca crème.

This was sterilized by heating it repeatedly, care being taken that it was not heated enough to cause any alteration in color. This was injected under the skin on the outside of the ears of the rabbits, the fluid being forced in under slight pressure until the tissues were permeated for some distance around the needle. At various times bits of the permeated tissues were excised and immediately fixed in mercuric chloride, imbedded in paraffin, and sectioned. In all cases the tissues still contained Scharlach R., and in most cases oil was still present. A summary of the results follows.

Experiment 1.—Young rabbit No. 1. Seven days after injection. The ear about the point of injection was swollen and congested. Section showed spaces in the connective tissue made by oil droplets; many hair follicles cut at various depths, some

extending almost to the cartilage, and many sebaceous glands. There was no evidence of proliferation in any of the epithelial structures, neither typical nor atypical mitoses, nor any evidence of an inflammatory reaction on the part of the connective tissue.

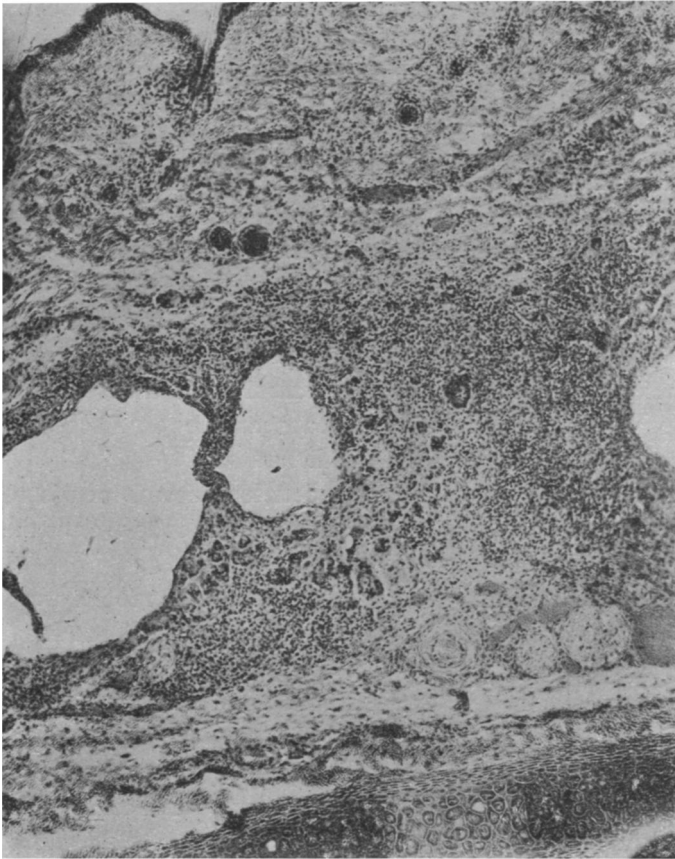


FIG. 1.—Section from ear of young rabbit 28 days after injection with scarlet oil. Note the marked inflammatory reaction, the large plasmodial masses surrounding the open spaces, the outlines of the giant cells not being made out. There is no epithelial proliferation.

Experiment 2.—Young rabbit No. 2. Twelve days after injection. The ear about the area of injection was very slightly, if at all, swollen, and somewhat congested. Section showed congestion, edema and slight mononuclear infiltration of the connective tissue. There was no evidence of epithelial proliferation, no mitoses, typical or atypical.

Experiment 3.—Old rabbit No. 4. Seventeen days after injection. The ear showed no pathological changes except redness from the presence of the dye. Section

showed large open spaces in the connective tissue which had been occupied by oil droplets, the connective tissue was edematous and showed slight mononuclear infiltration. There was no evidence of epithelial proliferation, neither typical nor atypical mitoses.

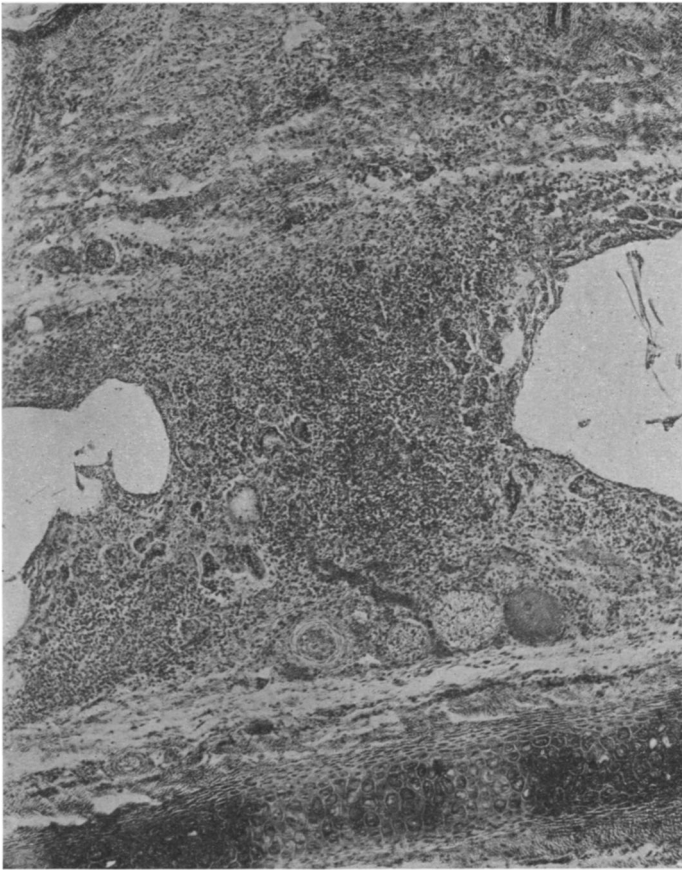


FIG. 2. — Same as Fig. 1. Higher magnification.

Experiment 4.—Small rabbit No. 2. Nineteen days after injection. The ear showed no pathological changes other than redness due to the dye. Section showed collapsed spaces made by oil droplets, about which there was hyperplastic connective tissue infiltrated with round cells, mainly plasma cells, and many multinucleated giant cells of the type of foreign-body giant cells. There was no evidence of proliferation on the part of the epithelium, neither typical nor atypical mitoses.

Experiment 5.—Old rabbit No. 4. Twenty-four days after injection. The ear showed only redness due to the dye. On section the connective tissue was very ede-

matous with slight mononuclear infiltration. There was no sign of epithelial proliferation, neither typical nor atypical mitoses.

Experiment 6.—Young rabbit No. 2. Twenty-eight days after injection. The ear showed only redness due to the presence of the dye. Section showed congestion,

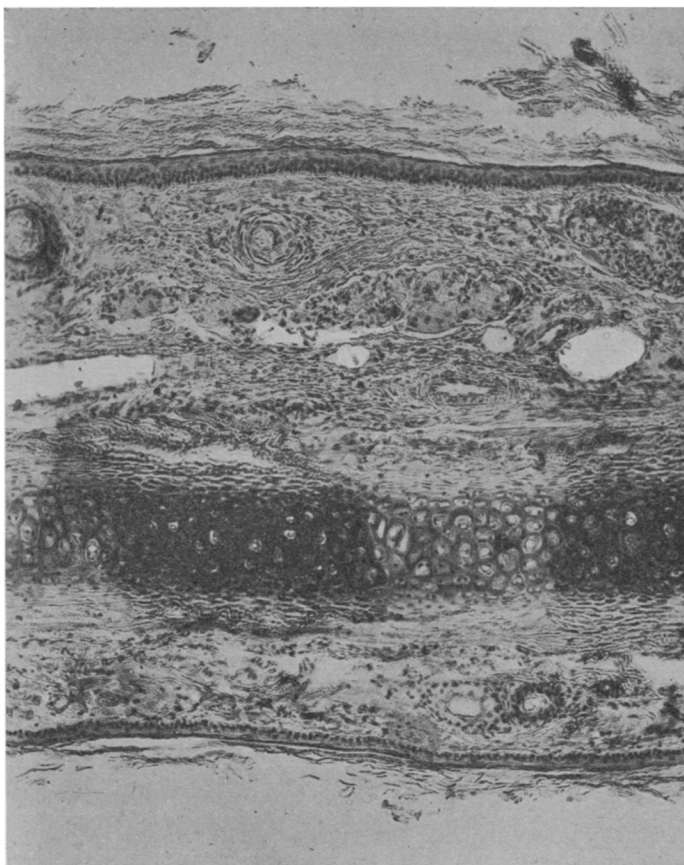


FIG. 3. — Section from ear of young rabbit 32 days after injection of scarlet oil, showing open spaces made by oil droplets, bordered by large plasmodial masses. Note slight inflammatory reaction and absence of epithelial proliferation.

hyperplasia of connective tissue, marked mononuclear infiltration and the presence of numerous open spaces made by oil droplets. Around these spaces there was a very marked infiltration of plasma cells and great numbers of giant cells of the character of foreign-body giant cells. In some places the walls of the open cavities consisted wholly of plasmodial masses of giant cells, the cell outlines not being visible. One of these giant cells showed a cell inclusion, the only evidence of phagocytosis seen in them.

The blood vessels about the spaces were congested. The dermis over them was thinned, but there was not the slightest evidence of proliferation on the part of the epithelial elements, either of the epidermis or of the hair follicles or of the glands. The only pathological change in the dermis was its slight thinning over the zone of reaction about

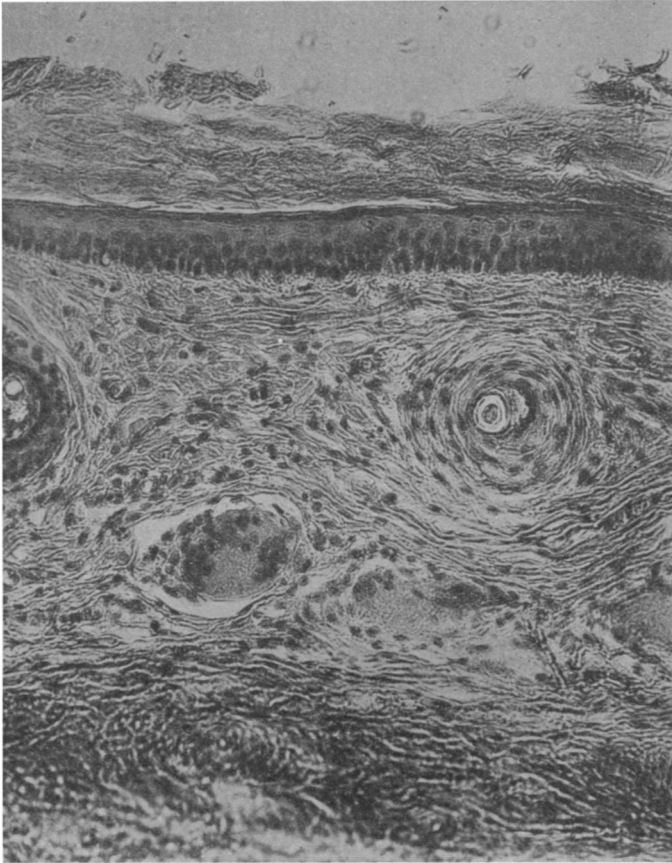


FIG. 4. — Same as Fig. 3. Higher power.

the spaces and an apparent increase in the number of wandering cells. There were no mitoses, either typical or atypical, in the epithelium and no signs of epithelial ingrowth.

Experiment 7.—Large rabbit No. 5. Thirty days after injection. The ear was red, due to the presence of dye. The sections showed open spaces surrounded by hyperplastic connective tissue, slight mononuclear infiltration, but the reaction about the spaces was very slight indeed when compared with that of the younger rabbit just

described. There was no evidence of epithelial proliferation. No mitoses, typical or atypical.

Experiment 8.—Small rabbit No. 3. Thirty-two days after injection. The ear showed only redness due to the presence of dye. The sections showed smaller

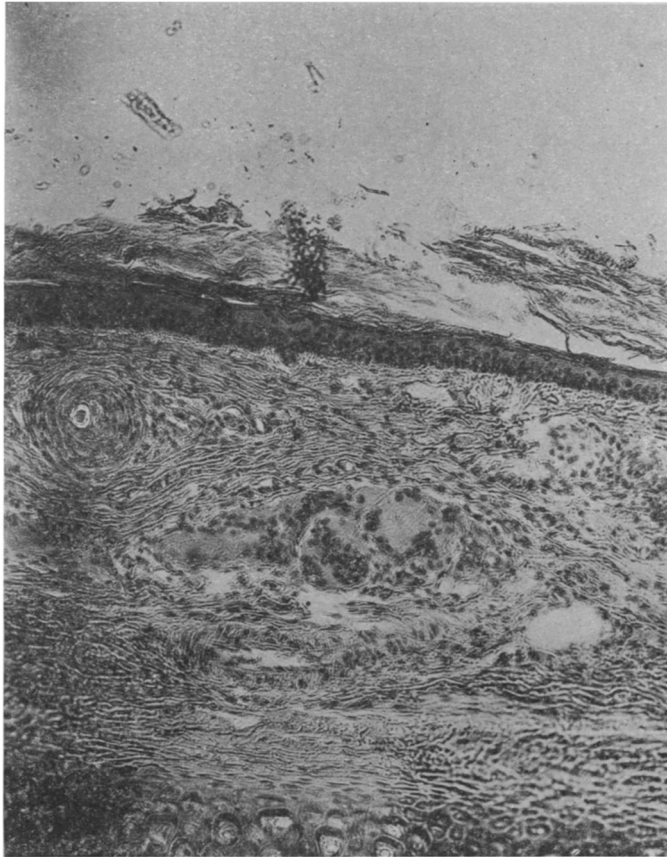


FIG. 5.—Same as Fig. 3. Higher magnification.

spaces than the previous sections, most of the oil having been absorbed, leaving behind the dye. About these smaller spaces the connective tissue was hyperplastic and infiltrated with plasma cells, the walls of the spaces being largely made up of very large multinucleated plasmodial masses of the character of foreign-body giant cells. There was no sign of epithelial proliferation, neither typical nor atypical mitoses.

Experiment 9.—Large rabbit No. 5. Forty-four days after injection. The ear showed only slight redness due to the dye. The sections showed large open spaces about which there was slight reaction, the connective tissue about them being hyperplas-

tic and slightly infiltrated with plasma cells. The spaces were lined by thin flattened endothelium. There was no evidence of epithelial proliferation.

Experiment 10.—Large rabbit No. 6. Sixty-one days after injection. The ear was slightly red, due to the presence of dye. It bore, though not near the site of injection,

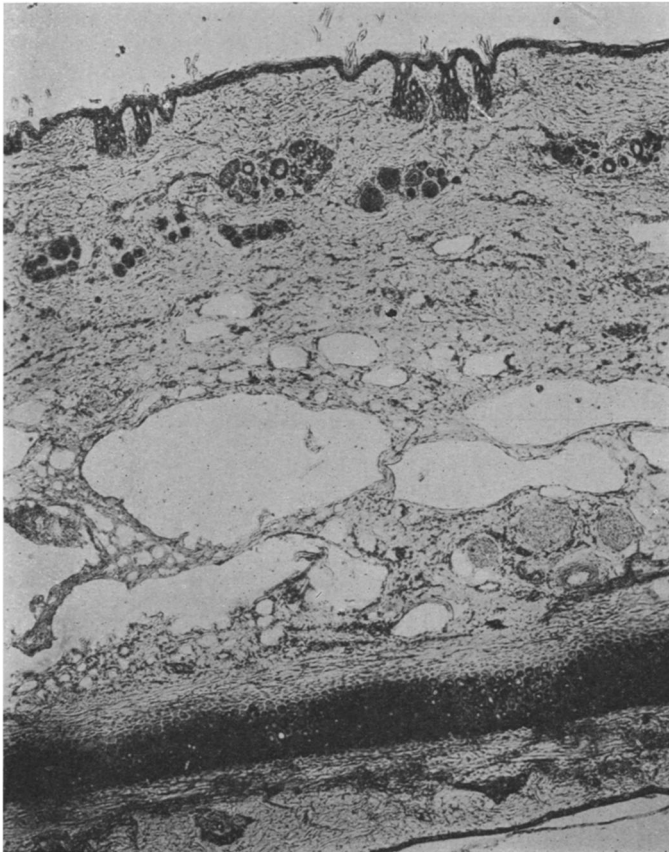


FIG. 6. — Section from ear of large rabbit 44 days after injection of scarlet oil, showing open spaces made by oil droplets. Note absence of inflammatory reaction and of epithelial proliferation.

a small indurated area. On section this proved to be a small sebaceous cyst. About this there was a slight inflammatory reaction, but the balance of the tissue showed no change aside from a hyperplasia of connective tissue. There was no evidence of epithelial proliferation, neither typical nor atypical mitoses.

These results show that the scarlet oil had no influence whatever on the epithelial cells, although it was present in the tissues at times

varying from seven to 61 days. It acted simply as a slight irritant, inducing a low-grade chronic inflammation. It is interesting to note that giant cells were formed as a result of the irritation only in the case of the young rabbits.

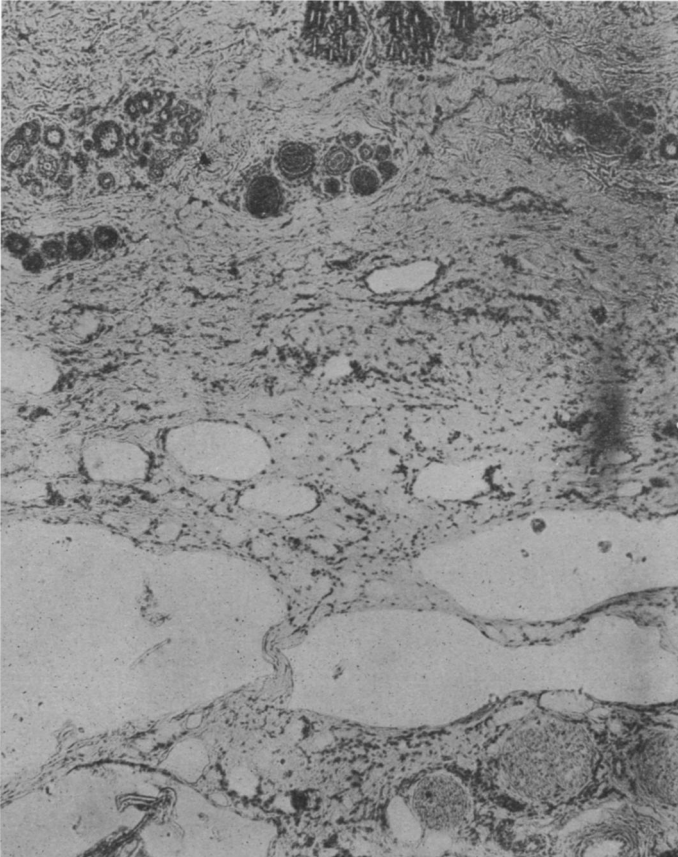


FIG. 7. — Same as Fig. 6. Higher power magnification.

So far as we can tell the work of Dr. Fischer has been duplicated. The character of injection and the time elements at least are identical, so far as we are able to tell from his paper, and yet our results are entirely negative. The question naturally arises, then, if Fischer's findings allow of any other explanation. Is it possible that he mistook foreign-body cells with inclusions for epithelial plugs?

As he does not mention the age or variety of the rabbits used we are in doubt as to whether this will prove to be the explanation. If he used only young rabbits such error might be possible, but this would

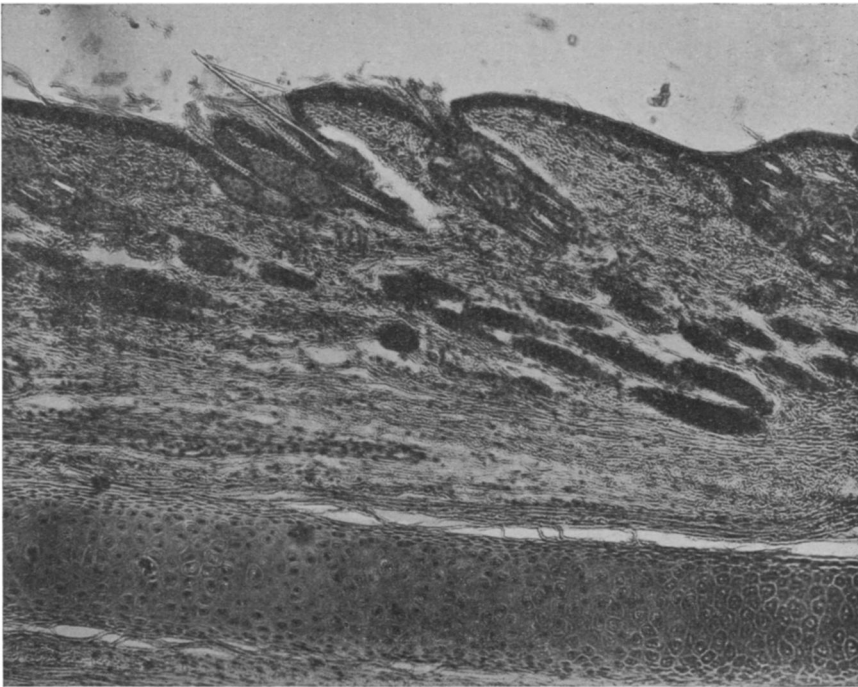


FIG. 8.—Section of rabbit's ear, normal, showing depth to which hairs penetrate. If the follicles were slightly hyperplastic, the picture might easily be mistaken for a squamous celled carcinoma.

not apply to old rabbits, for, as just pointed out, we found that foreign-body giant cells were not developed by the old animals.

There seems to remain only one other possibility, which we mention with hesitation, that physiological appearances were mistaken for pathological. Sections taken from different parts of the ear show great variation in the number of hairs and sebaceous glands and the depth to which these penetrate toward the cartilage. There would also be great variation between rabbits with smooth coats and those with rough coats. Larger hairs run obliquely at a slight angle for a long distance beneath the epithelium in some parts of the ear.

Oblique sections and even those at right angles will show in some areas normally a dermis set full of epithelial structures cut at various angles, longitudinally and transversely. In some cases the epithelia structures reach nearly, if not quite, to the cartilage. Fischer's Fig. 4 could easily be explained by such a section, as also his Figs. 1, 2, and 5. In Fig. 3 the upper part of the section could be normal structures cut obliquely, while the epithelium around the oil spaces might have been the foreign-body cells. Since Fischer's plates are made from drawings rather than from photographs, an absolute judgment is impossible.

We hesitate at offering such an explanation, and certainly do not wish to accuse Dr. Fischer of making such a gross error of judgment, but the difference in our results certainly calls for some explanation.

Further control work should be done along this line, inasmuch as my experiments seem to leave the attraxine theory on rather a weak foundation.

Dr. Warthin has controlled my work throughout and confirmed my observations. To him I am greatly indebted for this and for the great interest he has shown in the progress of the work.

NOTE.—After finishing the above work a similar set of experiments with Sudan III, Indo-phenol, and other similar substances was undertaken as a further control of Fischer's work. In the ear of a Belgian hare examined 12 days after injection of Sudan III in olive oil, a well-marked hyperplasia of the epithelium of the hair follicles over the area of injection was seen. Serial sections showed no atypical proliferation, the epithelium of the follicles showing only a simple hyperplasia. In the ear of a young, white rabbit injected at the same time with Sudan III in olive oil no hyperplasia was seen. This suggests the possibility that different varieties or species may react differently. Further work along these lines is under way and will be reported later.